

Increased Hepatocyte CYP2E1 Expression in a Rat Nutritional Model of Hepatic Steatosis With Inflammation

MARTIN D. WELTMAN,* GEOFFREY C. FARRELL,* and CHRISTOPHER LIDDLE*

*Storr Liver Unit, Department of Gastroenterology and Hepatology, and *Department of Clinical Pharmacology, University of Sydney at Westmead Hospital, Westmead, New South Wales, Australia

Background & Aims: Nonalcoholic steatohepatitis is morphologically identical to alcoholic hepatitis and has multiple etiologic associations and an unknown pathogenesis. The present study used a rat nutritional model of hepatic steatosis with inflammation to test the hypothesis that induction of the alcohol-inducible hepatic cytochrome P450 (CYP) 2E1 is associated with production of steatohepatitis. **Methods:** Rats received a diet devoid of methionine-choline. CYP2E1 protein was detected in liver sections by immunohistochemistry and in hepatic microsomal fractions by immunoblotting; CYP2E1 activity was detected by *N*-demethylation of *N,N*-dimethylnitrosamine (NDMA). CYP2E1 messenger RNA was analyzed by Northern blotting and slot blot hybridization. **Results:** After 4 weeks of methionine-choline devoid diet, macrovesicular steatosis and an inflammatory infiltrate were prominent in hepatic acinar zone 3. CYP2E1 immunostaining was increased and had a more extensive acinar distribution corresponding to that of the steatosis. Microsomal CYP2E1 protein, NDMA activity, and hepatic CYP2E1 messenger RNA levels were all correspondingly increased. **Conclusions:** CYP2E1 is induced, partly at a pretranslational level, in this experimental form of steatohepatitis. The finding of biochemical and histological similarities between this nutritional model of hepatic steatosis with inflammation and alcoholic hepatitis indicates possible clues to common pathogenetic mechanisms. The relevance of this finding to human nonalcoholic steatohepatitis remains uncertain and requires further investigation of human liver specimens.

The morphological features of steatohepatitis occur in several clinical settings. Although alcoholic liver disease is the most common cause,^{1,2} nonalcoholic steatohepatitis (NASH) is increasingly recognized as a significant form of liver disease.³⁻⁵ Traditionally, NASH has been described as a clinically mild entity with little progression,⁶⁻⁸ but it is now accepted that this disorder can progress to extensive fibrosis and cirrhosis.^{3,4,8} In the absence of a history of significant alcohol ingestion, steatohepatitis occurs most frequently in women who are

obese or have hyperlipidemia or diabetes mellitus^{1,3,9}; however, the association of lean men with NASH has recently been recognized.³ The morphological features of NASH include steatosis with focal necroinflammatory change, often including mononuclear and polymorphonuclear leukocytes, Mallory's bodies, and variable hepatic fibrosis.¹⁻³ Despite the morphological similarity to alcoholic steatohepatitis, little is known about the pathogenetic mechanisms involved in the development of NASH, particularly why such apparently diverse etiologies give rise to the same histological features.

In contrast to NASH, the pathology and pathogenesis of alcoholic hepatitis have been well studied. In both animals and humans, ethanol induces hepatic cytochrome P450 2E1 (CYP2E1).¹⁰⁻¹² Thus, CYP2E1 expression is increased in alcoholic hepatitis¹⁰ and has been suggested to contribute to the pathogenesis of this disorder through the generation of reactive oxygen species that cause cellular injury of the liver.¹¹⁻¹⁷ In both human and rat liver, CYP2E1 is expressed almost exclusively in acinar zone 3, and the induction by alcohol is also restricted to this region.¹⁸ CYP2E1 is also up-regulated in other clinical settings that are associated with steatohepatitis, such as diabetes mellitus and obesity.^{1,19-21}

In an earlier study in patients with NASH, it was noted that antipyrine metabolism was disproportionately reduced compared with other indices of liver function.³ This led to the suggestion that NASH is associated with destruction or down-regulation of cytochrome P450 enzymes. However, to our knowledge, a possible pathogenetic relationship between altered hepatic cytochrome P450 levels or expression of CYP2E1 and NASH has not been studied. The aim of the present study was to determine hepatic distribution, content, and catalytic activity of CYP2E1 in a rat nutritional model of hepatic

Abbreviations used in this paper: MCD, methionine-choline devoid; NASH, nonalcoholic steatohepatitis; NDMA, *N*-demethylation of *N,N*-dimethylnitrosamine; dCTP, deoxycytidine triphosphate.

© 1996 by the American Gastroenterological Association
0016-5085/96/\$3.00

macrovesicular steatosis with inflammation and cellular injury (steatohepatitis) to test the hypothesis that increased hepatic expression of this enzyme is involved in the pathogenesis of NASH. A diet devoid of methionine-choline (MCD diet) was used to produce fatty liver disease, in which other investigators have previously observed steatohepatitis.²²

Materials and Methods

Diet and Animals

The study was approved by the Western Sydney Area Health Service Animal Care and Ethics Committee. Adult male Wistar rats weighing 250–350 g obtained from the Department of Animal Care, Westmead Hospital, were fed an MCD diet (ICN Biomedicals, Sydney, Australia) for 4 or 13 weeks. Animals assigned to control groups (see below) received the same diet with the addition of choline bitartrate (2 g/kg) and DL-methionine (3 g/kg). Rats were maintained on a 12-hour light/dark cycle, under conditions of constant temperature (22°C) and humidity, and given free access to food and drinking water up until the time of killing. The rats were divided into two experimental groups: MCD diet and pair-fed isocaloric control diet. Additionally, a group of animals was fed a control diet ad libitum to compare weight and hepatic histology with the two experimental groups.

Tissue Preparation

At the end of the defined period, rats were anesthetized with ether and underwent venesection. The livers were removed and weighed. Tissue samples were taken from the medial lobe for histology, and portions were snap-frozen in liquid nitrogen for later RNA studies. Washed microsomal fractions were prepared from the remaining liver using differential ultracentrifugation as previously described.²³ Microsomes were snap-frozen in liquid nitrogen and stored as suspensions at -70°C in 50 mmol/L potassium phosphate buffer, pH 7.4, containing 20% glycerol until required. Microsomal protein was assayed by the method of Lowry et al.²⁴ using bovine serum albumin as the standard.

Chemicals

DL-Methionine and choline bitartrate were purchased from Sigma-Aldrich (Sydney, Australia). [4-¹⁴C]testosterone (sp act, 56 mCi/mmol) was a product of Amersham Australia (Sydney, Australia). Steroid standards were supplied by Sigma-Aldrich, Steraloids Inc. (Wilton, NH) or the MRC Steroid Reference Collection at Queen Mary's College (London, England). Silica gel thin-layer chromatographic plates containing F254 indicator (E. Merck, Darmstadt, Germany) were used for the separation of testosterone metabolites. Histo-Clear was purchased from National Diagnostics (Atlanta, GA), mounting medium from Fronine (Sydney, Australia), and polylysine-coated slides from Lomb Scientific (Sydney, Australia). All other chemicals, including the reagents for electrophoresis and

immunohistochemistry, were of analytical grade or better and were purchased from Sigma-Aldrich.

Biochemical and Other Assays

Assays for serum alanine aminotransferase, cholesterol, and triglyceride were performed in the Department of Clinical Chemistry, Insritute of Clinical Pathology and Medical Research at Westmead Hospital, using automated procedures. Serum concentrations of testosterone were estimated by commercial direct radioimmunoassay kit (CIS, Gif Sur Yvette, France). Microsomal cytochrome P450 content was determined according to the method of Omura and Sato²⁵ with sodium dithionite as reductant. P450 reductase activity was assayed as the reduced nicotinamide adenine dinucleotide phosphate-dependent rate of reduction of cytochrome c according to Gnospelious et al.²⁶ The method of Peng et al.²⁷ was used to determine the CYP2E1 catalyzed *N*-demethylation of *N,N*-dimethylnitrosamine (NDMA). Microsomal testosterone hydroxylase assays were performed according to Waxman et al.²⁸ and metabolite formation was determined as described previously.²⁹

Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis and Immunoblotting

CYP2E1 protein was detected in microsomes from MCD diet–fed and control–fed rats by immunoblotting, which was performed as described previously.³⁰ The primary antibody used was a rabbit anti-rat CYP2E1 antibody (a gift from Prof. M. Ingelman-Sundberg, Karolinska Institute, Stockholm, Sweden). The blots were washed and protein visualized using enhanced chemiluminescence (Amersham, Sydney, Australia) according to the manufacturer's instructions. The exposed film was analyzed using a laser densitometer (LKB Ultrascan XL; LKB, Bromma, Sweden).

Histology and Immunohistochemistry

Sections were stained with H&E, reticulin, and Gomori's trichrome. Hepatic CYP2E1 was evaluated in liver sections by immunohistochemistry using an immunoperoxidase technique. Hepatic sections in paraffin (5–6 µm thick) were mounted on polylysine-coated glass slides, deparaffinized with Histo-Clear and ethanol, and incubated with H₂O₂ for 5 minutes to reduce endogenous peroxidase activity. Thereafter, immunohistochemistry was performed according to an established method.³¹ In brief, sections were incubated for 2 hours with rabbit anti-rat CYP2E1 antibody (see above) at a concentration of 164 µg/mL with the addition of goat serum (1:20) to block nonspecific binding. Sections were then incubated with anti-rabbit immunoglobulin G–biotin conjugate (Silenus, Melbourne, Australia) followed by streptavidin peroxidase conjugate (Silenus). Peroxidase activity was revealed by immersion in 3,3'-diaminobenzidine for 5 minutes. Sections were then counterstained with hematoxylin, dehydrated, and mounted in Ultramount (Histo-Labs, Sydney, Australia).

Quantitation of CYP2E1 Messenger RNA

The CYP2E1 oligonucleotide probe was a 30'-mer encompassing bases 774–803 of the published sequence.³² Oligonucleotide probes were end-labeled with [α -³²P]deoxycytidine triphosphate (dCTP) using terminal deoxynucleotidyl transferase (Promega Inc., Sydney, Australia) according to manufacturer's instructions. The probe was evaluated by Northern blotting using standard methods.³³ RNA samples from both MCD and control animals showed a single band with the expected size for CYP2E1 messenger RNA (mRNA). Assessment of the accuracy of RNA loading was estimated by hybridizing membranes with an [α -³²P]dCTP end-labeled oligonucleotide complementary to 18S ribosomal RNA.

Because the probe was specific for CYP2E1, a slot blotting technique was used to evaluate experimental samples. In brief, total RNA was extracted from 20-mg fragments of liver using a commercially available system (RNeasy; Qiagen Inc., Chatsworth, CA) following the manufacturer's instructions. RNA concentrations were determined spectrophotometrically. RNA samples (5 μ g) were denatured with a solution containing a final mixture of 50% formamide, 6% formaldehyde, and 1X 3-(N-morpholino)propanesulfonic acid and neutralized by the addition of an equal volume of 10X standard saline citrate. The samples were loaded onto a nylon membrane (Hybond N⁺, Amersham) using a slot blot apparatus (Bio-Rad, Richmond, CA). Prehybridization was performed in a solution containing 50 mmol/L N-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid/2-(2-hydroxy-1, 1-bis(hydroxymethyl)ethyl)amino)ethanesulfonic acid, 1 mol/L sodium chloride, 0.1% tetrasodium pyrophosphate (0.1 mg/mL), 1% sodium dodecyl sulfate, 10X Denhardt's solution, and 25% formamide at 45°C for 1.5 hours. The CYP2E1 oligoprobe was then added to a fresh aliquot of the prehybridization solution and hybridized overnight at 45°C. Membranes were washed twice in 2X standard saline citrate and 1% sodium dodecyl sulfate for 20 minutes and analyzed using a phosphorimager (Molecular Dynamics Inc., Sunnyvale, CA). The CYP2E1 oligonucleotide probe was removed from the membranes with 0.4 mol/L NaOH for 30 minutes at 45°C before reprobing with [α -³²P]dCTP end-labeled oligo d(T)₁₂ to control for even loading of mRNA.

Statistical Analysis

Data are expressed as means \pm SD. Data for MCD-fed rats and pair-fed controls were compared using the unpaired Student's *t* test (two-tailed) with the exception of animal body weights, which were compared at various time points using analysis of variance followed by post hoc analysis with the Bonferroni test. A *P* value of <0.05 was regarded as a significant difference between groups.

Results

Effect of MCD Diet on Liver Histology and Animal Growth

The MCD diet-fed animals lost weight compared with their isocaloric pair-fed controls and rats fed ad

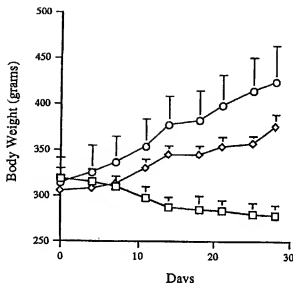


Figure 1. Body weight of rats during consumption of MCD diet (\square), rats isocalorically fed a control diet (\circ), and controls fed ad libitum (\triangle). Data are expressed as mean \pm SD for 6 rats in each group. Some error bars showing standard deviations have been omitted for clarity.

libitum; the weight loss was significant from day 7 ($P < 0.01$; Figure 1). Otherwise, the general condition of MCD diet-fed rats remained good throughout the experimental period. Liver sections from rats on the ad libitum control diet and from the isocaloric pair-fed controls had a normal morphological appearance (Figure 2A). In rats fed the MCD diet for 4 weeks, all livers had fatty infiltration, although this varied in severity and distribution. Macrovesicular steatosis was most prominent in acinar zone 3 (Figure 2B) and spread out in a panacinar distribution (but with periportal sparing) in the more severely affected livers. Similar findings were noted in the rats fed MCD diet for 13 weeks, but steatosis was more extensive and diffuse. In addition, there were numerous scattered foci of inflammation (Figure 2C), occasional hepatocyte necrosis, and some increase in perivascular fibrosis (Figure 2C).

Effect of MCD Diet on Serum Biochemical Parameters

Serum alanine aminotransferase level was significantly higher ($P < 0.001$) in the MCD diet-fed rats than in pair-fed controls, whereas serum cholesterol and triglyceride levels were decreased in the MCD diet-fed animals (Table 1). Serum testosterone level was significantly reduced in the MCD diet-fed group compared with the pair-fed rats (1.16 ± 0.17 vs. 3.75 ± 1.92 nmol/L; $P < 0.02$).

Immunohistochemistry for CYP2E1

In view of the increase in CYP2E1 protein and catalytic activity, the hepatic distribution of CYP2E1 was characterized using immunohistochemistry. In liver

sections from all pair-fed control rat livers, CYP2E1 immunostaining was localized exclusively in acinar zone 3 and confined to a layer of three to four cells around the terminal hepatic venule (Figure 3A). In the livers of

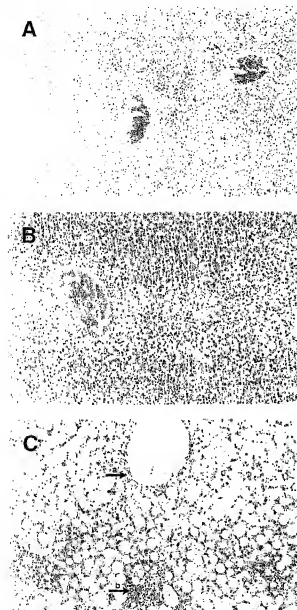


Figure 2. H&E-stained liver sections from rats fed the MCD diet and isocalorically paired controls. (A) Isocaloric pair-fed control rat (original magnification 100X). The histological appearances are normal. (B) A rat fed the MCD diet for 4 weeks (original magnification 100X). Fatty change is most prominent in zone 3. (C) A rat fed the MCD diet for 13 weeks (original magnification 250X). In addition to extensive fatty infiltration, fibrosis around the terminal hepatic venule (arrows), hepatocytic necrosis, and a prominent inflammatory infiltrate (arrow b) can be seen.

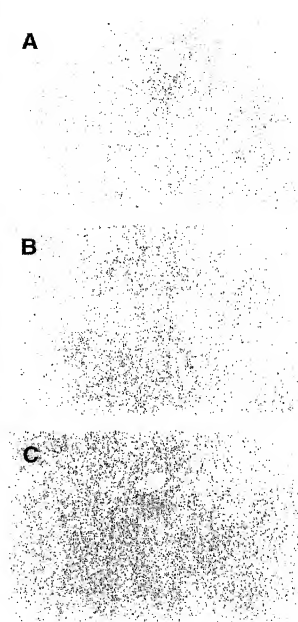


Figure 3. Immunostaining for CYP2E1 in liver sections from rats after 4-week intake of the MCD diet and pair-fed controls. (A) Pair-fed control rat (original magnification 100X). CYP2E1 immunostaining is confined to the hepatocytes that surround the terminal hepatic venule. (B and C) MCD diet-fed rat (original magnification 100X). Immunostaining for CYP 2E1 shows a more diffuse pattern of staining throughout zone 3 and extending into zone 2. The immunostaining for CYP2E1 corresponds to the distribution of steatosis.

Table 1. Serum Biochemical Indices in Rats Fed an MCD Diet and in Their Isocalorically Pair-Fed Controls After 4 Weeks

Experimental group	n	ALT (U/L)	Cholesterol (mmol/L)	Triglyceride (mmol/L)	Testosterone (nmol/L)
MCD	6	114 ± 6*	1.5 ± 0.1*	0.7 ± 0.1*	1.2 ± 0.2*
Isocaloric control	6	27 ± 2	1.8 ± 0.2	2.5 ± 0.4	3.8 ± 1.9

NOTE. The results are expressed as mean ± SD.

ALT, alanine aminotransferase.

* $P < 0.001$, * $P < 0.02$; significant difference from control.* $P = 0.058$, not significant.

MCD diet-fed rats, the pattern of 2E1 immunostaining closely followed the distribution of steatosis; it extended more diffusely through zone 3 than in pair-fed controls, extending to zone 2 in some cases (Figure 3B and 3C). This finding was found consistently in liver sections from all 6 MCD diet-fed animals.

Effect of MCD Diet on Hepatic Microsomal Total P450 Content, P450 Reductase, and Activities of CYPs 2C11, 3A2, 2A1, and 2E1

Total microsomal cytochrome P450 content in the livers of rats fed the MCD diet decreased to 70% of that of the pair-fed controls at the end of 4 weeks ($P < 0.05$; Table 2). P450 reductase activity was increased at the end of 4 weeks in the MCD diet-fed animals compared with the pair-fed controls ($P < 0.002$; Table 2).

To determine the nature of the decrease in total cytochrome P450, changes in individual CYP enzymes were assessed using a profile of microsomal catalytic activities (Table 2). Pathways of testosterone hydroxylation mediated by CYP2C11 (at the 2 α and 16 α positions), CYP3A2 (at the 6 β position), and CYP2A1 (at the 7 α position) were decreased in the MCD diet group compared with pair-fed controls ($P < 0.001$). This was most pronounced for CYP2C11, with catalytic activity reduced to 20% of control at week 4, whereas catalytic activity for CYP3A2 and CYP2A1 was reduced to 56%

and to 63%, respectively, at week 4. In contrast, the catalytic activity of CYP2E1 in hepatic microsomes, measured as NDMA, increased twofold in MCD diet-fed rats compared with the respective pair-fed controls (Figure 4).

Effect of MCD Diet on Hepatic Microsomal CYP2E1 Protein Content

An increase in staining intensity was observed when microsomal proteins from MCD diet-fed animals were probed with anti-CYP2E1 antibody (Figure 5). Densitometric scanning of these immunoblots showed a threefold increase in CYP2E1 protein content in the MCD diet group fed for 4 weeks compared with pair-fed controls ($P < 0.001$; Figure 4).

Analysis of CYP2E1 mRNA

Slot blot analysis of hepatic RNA samples showed a 2.5-fold increase in hepatic CYP2E1 mRNA concentrations in the livers of MCD diet-fed rats compared with their pair-fed controls (4440 ± 1630 vs. 1440 ± 420 arbitrary densitometry units; $P < 0.001$) at the end of 4 weeks of feeding (Figures 4 and 6). This increase in CYP2E1 mRNA paralleled the increase in CYP2E1 protein and catalytic activity.

Discussion

The important finding of this study is that, in a nutritional model of steatohepatitis, the hepatic micro-

Table 2. Microsomal P450 Content and P450 Reductase and Testosterone Hydroxylation Activities in Hepatic Microsomes From MCD Diet-Fed and Isocalorically Pair-Fed Control Rats After 4 Weeks

Experimental group	n	Microsomal P450 (nmol/mg)	P450 reductase (nmol · mg ⁻¹ · min ⁻²)	Testosterone hydroxylation pathway (product · min ⁻¹ · mg microsomal protein ⁻¹)			
				16 α	2 α	7 α	6 β
MCD	6	0.62 ± 0.04*	365 ± 46*	0.39 ± 0.10*	0.20 ± 0.15*	0.20 ± 0.02*	1.13 ± 0.26*
Isocaloric control	6	0.89 ± 0.17	220 ± 45	2.02 ± 0.34	1.65 ± 0.19	0.31 ± 0.01	2.04 ± 0.14

NOTE. The results are expressed as mean ± SD.

* $P < 0.001$, * $P < 0.05$, * $P < 0.002$; significant difference from control.* $P = 0.06$, not significant.

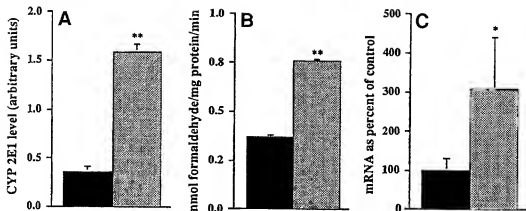


Figure 4. (A) CYP2E1 protein, (B) NDMA activity, and (C) CYP2E1 mRNA in MCD diet-fed rats (■) and isocaloric pair-fed controls (■) after 4 weeks of dietary intake. * $P < 0.001$ and ** $P < 0.0001$, MCD diet-fed rats compared with pair-fed controls. Results are expressed as mean \pm SD.

somal content of CYP2E1 was significantly increased. The increased hepatic CYP2E1 content was catalytically active, as shown by the enhancement of microsomal NDMA metabolism. Furthermore, the finding of increased hepatic content of CYP2E1-specific mRNA indicated that pretranslational mechanisms were largely operative for the increased CYP2E1 expression. The substantial decrease in total P450 content and the decreased catalytic activities of other CYP proteins that are normally expressed at high levels in male rat liver further emphasize the significance of the enhanced CYP2E1 up-regulation in this model.

The MCD diet consistently induced not only hepatic steatosis but also an associated inflammatory infiltrate. It therefore seems to be a simple and reproducible animal model of fatty liver disease with inflammation and cellular injury that morphologically resembles the group of human disorders known as NASH. In rats, starvation has been reported to cause hepatic steatosis.² The possibility that caloric deprivation was the cause of liver disease in MCD diet-fed animals was considered unlikely in the present study because normal liver histology was found in isocalorically pair-fed controls, although these pair-fed controls grew more slowly than the rats fed ad libitum. The fact that the MCD diet-fed rats actually lost weight indicates the operation of mechanisms other than caloric deprivation. One likely explanation is that the hepatic inflammation induced by this diet caused a catabolic state mediated by cytokines, such as tumor necrosis factor α .³⁴ The weight loss documented in the MCD diet-fed animals has also been observed by other investigators.^{35,36} In addition, the observed elevation of serum alanine aminotransferase concentration is consistent with

the hepatic necroinflammatory process observed histologically in the MCD diet-fed rats. The low serum lipid values found in the MCD diet-fed rats exclude hyperlipidemia, particularly hypertriglyceridemia, as a possible cause of steatohepatitis in this model; however, NASH has also been observed in humans with normal serum lipid values.³

As documented in alcoholic steatohepatitis,¹ the early stages of liver disease in the MCD diet-fed rats were associated with steatosis mostly in acinar zone 3, with later extension into zones 2. Hepatocytes adjacent to the periportal region were consistently spared. CYP2E1 is normally localized within a rim of hepatocytes three to four cells wide around the terminal hepatic venule in acinar zone 3.^{11,18,31} In rats, induction of CYP2E1 by prolonged alcohol consumption occurs in the same locality, with a larger number of cells in acinar zone 3 express-

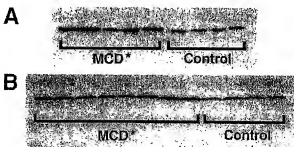


Figure 5. Immunoblot for CYP2E1 in hepatic microsomal proteins from MCD diet-fed and pair-fed control rats after (A) 4 weeks and (B) 13 weeks of dietary intake. At both time points, intensity of immunostaining in microsomes from MCD diet-fed rats is greater than that of pair-fed controls (* $P < 0.001$).



Figure 6. Slot blot analysis of CYP2E1 mRNA in pair-fed controls (C) and MCD diet-fed rats (MCD) after 4 weeks of dietary intake. There is a greater intensity of CYP2E1 mRNA expression in the MCD diet-fed rats than in their ad libitum-fed controls (4440 ± 1630 and 1440 ± 420 arbitrary units; * $P < 0.001$).

ing CYP2E1.^{11,31} Moreover, a characteristic histological feature of alcoholic liver injury is the predominance of lesions in acinar zone 3.^{11,33,37} Thus, the induction of CYP2E1 in MCD diet-fed rats is strikingly similar to that found in alcoholic steatohepatitis and also follows the distribution of steatosis.

The increased CYP2E1 mRNA levels observed in MCD diet-fed rats indicate that induction of CYP2E1 appears, at least in part, to be at the pretranslational level. This finding is different from a study on alcoholic liver disease in which the mechanism of CYP2E1 induction was largely posttranslational through protein stabilization.³⁸ It is, however, similar to that documented in rats fed a high-fat diet,³⁹ in fasting rats,⁴⁰ and in diabetic animals.⁴¹ In each of these situations, the increases in CYP2E1 mRNA levels mirror the increased CYP2E1 protein and catalytic activity. Recent studies indicate that under certain conditions, pretranslational induction of CYP2E1 by ethanol can also occur.^{42,43} The association of hepatic CYP2E1 induction with obesity, diabetes, and fasting in rats is of potential relevance to human disease because these factors have been associated with NASH in humans.²

Induction of CYP2E1 probably plays a central role in the production of cellular injury in alcoholic hepatitis.^{11,12,44,45} CYP2E1-catalyzed ethanol oxidation results in the formation of free radicals capable of peroxidizing cell membranes.¹³ It has therefore been suggested that the elevated CYP2E1 levels found in zone 3 hepatocytes after long-term alcohol intake lead to increased production of ethanol-derived toxic metabolites.^{44,45} In turn, this would be expected to enhance the susceptibility of hepatocytes in this location to alcohol-mediated damage.¹³ Because the increased CYP2E1 protein level associated with intake of the MCD diets was catalytically active, it is likely that similar CYP2E1-mediated processes

could generate reactive oxygen species and other radicals in this nutritional model of steatohepatitis.

The increase in CYP2E1 activity observed after intake of the MCD diet was all the more remarkable because of the decrease in total P450 content. Our findings differ from those of a rat model of long-term alcohol ingestion in which total P450 content was increased,⁴⁶ but they are consistent with the clinical finding that patients with NASH have a decrease in oxidative drug metabolism that is disproportionate to other indices of hepatic function.⁵ In the MCD diet model, the reduction in total cytochrome P450 content detected in the livers of MCD diet-fed animals was mainly caused by the observed decrease in CYPs 2C11 and 3A2, which together constitute more than 50% of total hepatic cytochrome P450 content of the adult male rat.⁴⁶ Testosterone is required for the expression of CYPs 2C11 and 3A2.⁴⁷ Thus, the decline in catalytic activities of these hepatic proteins is probably partly caused by the reduction in serum testosterone levels detected in the MCD diet-fed animals. The elevated CYP2E1 activity and protein levels in the presence of reduced activities of these CYPs suggest that CYP2E1 assumes a more significant proportion of the total P450 content in the liver of MCD diet-fed rats.

In conclusion, rats fed an MCD diet developed fatty liver disease with hepatic inflammation, a form of liver disease that is morphologically identical to NASH in humans. In this model of steatohepatitis, CYP2E1 protein levels and activity were elevated, thereby resembling alcoholic steatohepatitis^{11,31} in the same way as the centrilobular distribution of steatosis and CYP2E1 expression.^{11,37} The similar changes in CYP2E1 in this nutritional model of hepatic steatosis with inflammation and alcoholic steatohepatitis imply that these two disorders share pathogenic mechanisms. The relevance of this finding to human NASH remains uncertain and requires further investigation of human liver specimens.

References

- Burt AD, MacSween RNM, Peters TJ, Simpson KJ. Non-alcoholic fatty liver: causes and complications. In: McIntyre N, Benhamou JP, Burcher J, Rizzetto M, Rodes J, eds. Oxford textbook of clinical hepatology. Volume 2. 1st ed. Oxford: Oxford University, 1991: 865-871.
- Zimmerman HJ, Ishak KG. Non-alcoholic steatohepatitis and other forms of pseudoalcoholic liver disease. In: Hall P, ed. Alcoholic liver disease: pathology and pathogenesis. 2nd ed. London: Edward Arnold, 1994:175-198.
- Bacon BR, Farahvash MJ, Janney CG, Neuschwander-Tetri BA. Nonalcoholic steatohepatitis: an expanded clinical entity. Gastroenterology 1994;107:1103-1109.
- Powell EE, Cooksley WGE, Hanson R, Searle J, Halliday JW, Powell LW. The natural history of non-alcoholic steatohepatitis: a

- follow-up study of forty-two patients for up to 21 years. *Hepatology* 1990;11:74-80.
5. Fiatarone JR, Coverdale SA, Batey RG, Farrell GC. Non-alcoholic steatohepatitis: impaired antipyrine metabolism and hypertriglyceridaemia may be clues to its pathogenesis. *J Gastroenterol Hepatol* 1991;6:585-590.
 6. Ludwig J, Viggiano TR, McGill DB, Ott BJ. Non-alcoholic steatohepatitis. Mayo Clinic experience with a hitherto unnamed disease. *Mayo Clin Proc* 1980;55:434-438.
 7. Eriksson S, Eriksson KF, Bondesson L. Non-alcoholic steatohepatitis in obesity: a reversible condition. *Acta Med Scand* 1986;220:83-88.
 8. Itoh S, Yougel T, Kawagoe K. Comparison between non-alcoholic steatohepatitis and alcoholic hepatitis. *Am J Gastroenterol* 1987;82:650-654.
 9. Wanless IR, Lentz JS. Fatty liver hepatitis (steatohepatitis) and obesity: an autopsy study with analysis of risk factors. *Hepatology* 1990;12:1106-1110.
 10. Ingelman-Sundberg M, Ekström G, Tindberg N. Lipid peroxidation dependent on ethanol-inducible cytochrome P-450 from rat liver. *Adv Biophys* 1988;71:43-47.
 11. Takahashi T, Lasker JM, Rosman AS, Lieber CS. Induction of cytochrome P-4502E1 in the human liver by ethanol is caused by a corresponding increase in encoding messenger RNA. *Hepatology* 1993;17:236-245.
 12. Ingelman-Sundberg M, Johansson I, Vin H, Terelius Y, Eliasson E, Clot P, Albano E. Ethanol-inducible cytochrome P450 2E1: genetic polymorphism, regulation and possible role in the etiology of alcohol-induced liver disease. *Alcohol* 1993;10:447-452.
 13. Persson JO, Terelius Y, Ingelman-Sundberg M. Cytochrome P450 dependent formation of reactive oxygen radicals: Isoenzyme-specific inhibition of P450 mediated reduction of oxygen and carbon tetrachloride. *Xenobiotica* 1990;20:887-900.
 14. Ekström G, Ingelman-Sundberg M. Rat liver microsomal NADPH-supported oxidase activity and lipid peroxidation dependent on ethanol-inducible cytochrome P450 (P-450IIE1). *Biochem Pharmacol* 1989;38:1313-1319.
 15. Dai Y, Rashba Step J, Cederbaum AL. Stable expression of human cytochrome P4502E1 in HepG2 cells: characterization of catalytic activities and the production of reactive oxygen intermediates. *Biochemistry* 1993;32:6928-6937.
 16. Gorsky LD, Koop DR, Coon MJ. On the stoichiometry of the oxidase and monooxygenase reactions catalysed by liver microsomal cytochrome P450. *J Biol Chem* 1984;259:6812-6817.
 17. Koop DR. Oxidative and reductive metabolism by cytochrome P-450 2E1. *FASEB J* 1992;6:724-730.
 18. Bühler R, Lindros KO, von Boguslawsky K, Kärkkäinen P, Mäkinen J, Ingelman-Sundberg M. Perivenous expression of ethanol-inducible cytochrome P450IIE1 in livers from alcoholics and ethanol-fed rats. *Alcohol Alcoholism* 1991;1:311-315.
 19. Favreau LV, Malchoff DM, Mole JE, Schenkenman JB. Responses to insulin by two forms of rat hepatic microsomal cytochrome P-450 that undergo major (RLM6) and minor (RLM5b) elevations in diabetes. *J Biol Chem* 1987;262:14319-14326.
 20. Raucy JL, Lasker JM, Kraner JC, Salazar DE, Lieber CS, Corcoran GB. Induction of cytochrome P450IIE1 in the obese overfed rat. *Mol Pharmacol* 1991;39:275-280.
 21. Dong Z, Hong J, Ma Q, Li D, Bullock J, Gonzalez FJ, Park SS, Gelboin HV, Yang CS. Mechanism of induction of cytochrome P450ac (P450) in chemically induced and spontaneously diabetic rats. *Arch Biochem Biophys* 1988;263:29-35.
 22. Teramoto K, Bowers JL, Khetry U, Palombo JD, Clouse ME. A rat fatty liver transplant model. *Transplantation* 1993;55:737-741.
 23. Murray M, Zaluzny L, Farrell GC. Drug metabolism in cirrhosis. Selective changes in cytochrome P-450 isoenzymes in the choline-deficient rat model. *Biochem Pharmacol* 1986;35:1817-1824.
 24. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement by the Folin phenol reagent. *J Biol Chem* 1951;193:265-275.
 25. Omura T, Sato R. The carbon monoxide binding pigment of liver microsomes: solubilization, purification and properties. *J Biol Chem* 1964;239:2379-2385.
 26. Gnosspelius Y, Thor H, Orenius S. A comparative study on the effects of phenobarbital and 3,4-benzpyrene on the hydroxylating enzyme system of rat-liver microsomes. *Chem Biol Interact* 1969;1:125-137.
 27. Peng R, Tennant P, Lorr NA, Yang CS. Alterations of microsomal monooxygenase system and carcinogen metabolism by streptozotocin-induced diabetes in rats. *Carcinogenesis* 1983;4:703-708.
 28. Waxman DJ, Ko A, Walsh C. Regioselectivity and stereoselectivity of androgen hydroxylations catalyzed by cytochrome P-450 isoenzymes purified from phenobarbital-induced rat liver. *J Biol Chem* 1983;258:11937-11947.
 29. Murray M, Zaluzny L, Farrell GC. Impaired androgen 16 alpha-hydroxylation in hepatic microsomes from carbon tetrachloride-cirrhotic male rats. *Gastroenterology* 1987;93:141-147.
 30. Cantrill E, Murray M, Mehta I, Farrell GC. Down regulation of the male-specific hepatic microsomal steroid 16 alpha-hydroxylase, cytochrome P450 UTA, in rats with portal bypass: relevance to estradiol accumulation and impaired drug metabolism in hepatic cirrhosis. *J Clin Invest* 1989;83:1211-1216.
 31. Ingelman-Sundberg M, Johansson I, Penttilä KE, Glaumann H, Lindros KO. Centrilobular expression of ethanol-inducible cytochrome P450 (IIE1) in rat liver. *Biochem Biophys Res Commun* 1988;157:55-60.
 32. Song BJ, Gelboin HV, Park S-S, Yang CS, Gonzalez FJ. Complementary DNA and protein sequences of ethanol-inducible rat and human cytochrome P-450s. *J Biol Chem* 1986;261:16689-16697.
 33. Chen J, Murray M, Liddle C, Jiang X-M, Farrell GC. Downregulation of male-specific cytochrome P450s 2C11 and 3A2 in bile duct-ligated male rats: importance to reduced hepatic content of cytochrome P450 in cholestasis. *Hepatology* 1995;22:580-587.
 34. Tracey KJ, Cerami A. Metabolic responses to cachectin/TNF. A brief review. *Ann NY Acad Sci* 1990;587:325-331.
 35. Fullerton FR, Greenman DL, Blydes BS, Polier LA. Ethynylestradiol protection against methyl insufficiency in castrated male Wistar/Furth rats fed a methionine-choline-deficient diet. *Carcinogenesis* 1993;14:1237-1240.
 36. Brkwith MH, Anderson D. An evaluation of two animal models for dietary influences on reproductive toxicology. *In Vivo* 1991;5:153-157.
 37. Morimoto M, Hagbjörk A, Wan Y-Y, Fu PC, Clot P, Albano E, Ingelman-Sundberg M, French SW. Modulation of experimental alcohol-induced liver disease by cytochrome P450 2E1 inhibitors. *Hepatology* 1995;21:1610-1617.
 38. Roberts BJ, Shoaf SE, Jeong K-S, Song BJ. Induction of CYP2E1 in liver, kidney, brain and intestine during chronic ethanol administration and withdrawal: evidence that CYP2E1 possesses a rapid phase half-life of 6 hours or less. *Biochem Biophys Res Commun* 1994;205:1064-1071.
 39. Yun YP, Casazza JP, Sohn DH, Veech RL, Song BJ. Pretransla-

- tional activation of cytochrome P45011E1 during ketosis induced by a high fat diet. *Mol Pharmacol* 1992;41:474-479.
40. Johansson I, Lindros K, Eriksson H, Ingelman-Sundberg M. Transcriptional control of CYP2E1 in the perivenous liver region and during starvation. *Biochem Biophys Res Commun* 1990;173:331-338.
41. Song BJ, Matsunaga T, Hardwick JP, Park SS, Veech RL, Gelboin HV, Gonzalez FJ. Stabilization of cytochrome P450J messenger ribonucleic acid in the diabetic rat. *Mol Endocrinol* 1987;1:542-547.
42. Ronis MJ, Huang J, Crouch J, Mercado C, Irby D, Valentine C, Lumpkin CK, Ingelman-Sundberg M, Badger TM. Cytochrome P450 2E1 induction during chronic alcohol exposure occurs by a two-step mechanism associated with blood alcohol concentrations in rats. *J Pharmacol Exp Ther* 1993;264:944-950.
43. Tsutsumi M, Lasker JM, Takahashi T, Lieber CS. In vivo induction of hepatic P4502E1 by ethanol: role of increased enzyme synthesis. *Arch Biochem Biophys* 1993;304:209-218.
44. Albano E, Clot P, Morimoto M, Tomasi A, Ingelman-Sundberg M, French SW. Role of cytochrome P4502E1-dependent formation of hydroxyethyl free radical in the development of liver damage in rats intragastrically fed with ethanol. *Hepatology* 1996;23:155-163.
45. French SW, Wong K, Albano E, Hagbjork AL, Ingelman-Sundberg M. Effect of ethanol on cytochrome P450 2E1 (CYP2E1), lipid peroxidation, and serum protein adduct formation in relation to liver pathology pathogenesis. *Exp Mol Pathol* 1993;58:61-75.
46. Waxman DJ, Dannan GA, Guengerich FP. Regulation of rat hepatic cytochrome P450: age-dependent expression, hormonal imprinting, and xenobiotic inducibility of sex-specific isoenzymes. *Biochemistry* 1985;24:4409-4417.
47. Waxman DJ. Interactions of hepatic cytochromes P-450 with steroid hormones: regioselectivity and stereospecificity of steroid metabolism and hormonal regulation of rat P-450 enzyme expression. *Biochem Pharmacol* 1988;37:71-84.

Received November 13, 1995. Accepted August 26, 1996.

Address requests for reprints to: Geoffrey C. Farrell, M.D., Department of Gastroenterology and Hepatology, Westmead Hospital, Westmead 2145, New South Wales, Australia. Fax: (61) 2-635-7592. e-mail: gfarrell@westmed.wk.nsw.edu.au.

Dr. Weltman is a recipient of an Australian National Health and Medical Research Council Postgraduate Medical Scholarship.

Dr. Farrell is the Robert W. Storr Professor of Hepatic Medicine at the University of Sydney.

Presented in part at Digestive Diseases Week, San Diego, May 13-18, 1995, and published in abstract form (*Gastroenterology* 1995;108:A1197).

The authors thank Prof. Magnus Ingelman-Sundberg (Karolinska Institutet, Stockholm, Sweden) for the gift of anti-rat CYP2E1 antibody and Bryan Goodwin for his expert technical assistance.